B16

(12) UK Patent Application (19) GB (11) 2 400 518 (13) A

(43) Date of Printing by UK Office 13.10.2004

(21) Application No: 0416718.5

(22) Date of Filing: 15.01.2003

(30) Priority Data:
(31) 0200938 (32) 16.01.2002 (33) GB

(86) International Application Data:
PCT/GB2003/000154 En 15.01.2003

(87) International Publication Data:
WO2003/062897 En 31.07.2003

(71) Applicant(s):
Solexa Limited
(Incorporated in the United Kingdom)
Chesterford Research Park,
Little Chesterford, NR SAFFRON WALDEN,
Essex, CB10 1XL, United Kingdom

(72) Inventor(s):
Mark Osborne
Jason Bryant

(continued on next page)

(51) INT CL[7]:
G02B 21/00 , G01N 21/64 , G02B 5/04

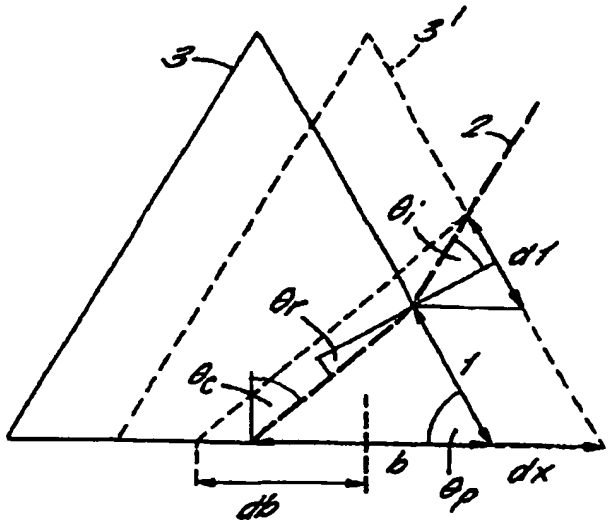
(52) UK CL (Edition W):
H4F FAAL FJS

(56) Documents Cited by ISA:
US 6327238 B1 US 6144498 A
US 5980831 A

(58) Field of Search by ISA:
INT CL[7] G01N, G02B
Other: EPO-Internal, WPI Data, PAJ

(54) Abstract Title: **Prism design for optical scanning applications**

(57) There is disclosed a prism for use in scanning applications such as total internal reflection microscopy in which the prism is translated relative to an incident light beam. A geometry is disclosed which cancels walk of the beam footprint at the base of the prism.



GB 2 400 518 A

GB 2400518 A continuation

(74) Agent and/or Address for Service:
Boult Wade Tennant
Verulam Gardens, 70 Gray's Inn Road,
LONDON, WC1X 8BT, United Kingdom

Original document

PRISM DESIGN FOR OPTICAL SCANNING APPLICATIONS

Patent number: WO03062897
Publication date: 2003-07-31
Inventor: OSBORNE MARK (GB); BRYANT JASON (GB)
Applicant: SOLEXA LTD (GB); OSBORNE MARK (GB); BRYANT JASON (GB)
Classification:
- international: ***G01N21/55; G02B5/04; G02B21/00; G02B26/10; G02B27/56;*** **G01N21/55; G02B5/04; G02B21/00; G02B26/10; G02B27/56;** (IPC1-7): G02B21/00; G02B5/04
- european:
Application number: WO2003GB00154 20030115
Priority number(s): GB20020000938 20020116

Also published as:
US2005057798 (A
GB2400518 (A)

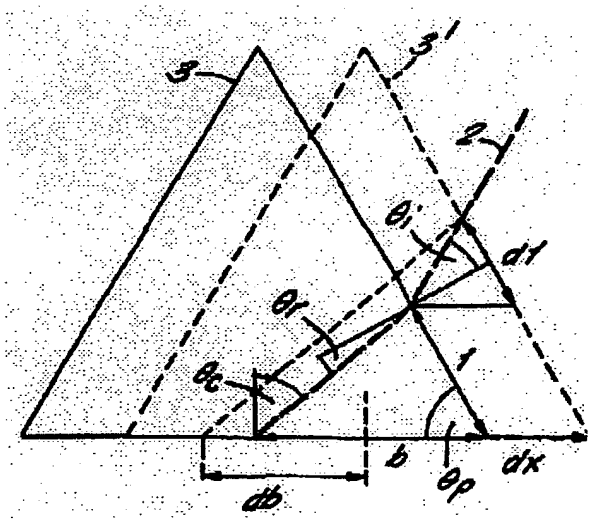
Cited documents:
US6327238
US5980831
US6144498

View INPADOC patent family

Report a data error he

Abstract of **WO03062897**

There is disclosed a prism for use in scanning applications such as total internal reflection microscopy in which the prism is translated relative to an incident light beam. A geometry is disclosed which cancels walk of the beam footprint at the base of the prism.



Description of **WO03062897**

<Desc/Clms Page number 1>

PRISM DESIGN FOR OPTICAL SCANNING APPLICATIONS

The present invention relates to scanning applications in which a sample, together with an angular optic a triangular or trapezoidal prism, are scanned in a fixed laboratory frame of reference. The fixed laborat frame is defined by a fixed light source. generating a fixed light beam and a detector. The angular optic couples the light beam to the base interface of the angular optic where the sample is located, such that th beam is incident on the base interface at an off normal angle. In such applications it is desirable that the intercept of the light beam at the base interface of the angular optic (the"footprint") remains stationary in the laboratory frame as the optic and sample are scanned so that there is no loss of image integrity at the detector.

One example of such an application is Total Internal Reflection Microscopy, which is a technique for observing samples illuminated by an evanescent wave. Total internal reflection occurs when a beam of light travelling through a very dense medium such as glass encounters an interface with a less dense medium such as air or water, at an angle to the normal which is greater than the critical angle for the interface. The critical angle for a glass/water interface is given by Fresnel's Law of Refraction as: S = si (nwater/nglass)

At angles greater than the critical angle, when total internal reflection takes place, an electric field component of the light penetrates through the interface into the water as an evanescent wave. The evanescent wave has the same wavelength as the incident beam but penetrates only a very short distance into the water, typically no more than 1Am. The evanescent wave decays exponentially from the interfa into the water with a characteristic

<Desc/Clms Page number 2>

penetration depth dependent.. on the wavelength and angle of incidence of the totally internally reflecte light.

In Total Internal Reflection Fluorescence Microscopy, fluorophores may be excited by the light in the evanescent field if they are close to the glass/water interface, but fluorophores further away in the bulk c the solution will. not be excited. The result is that images with very low background fluorescence are obtained. Figure 1 shows a typical instrument set up used in Total Internal Reflection Fluorescence Microscopy. A sample is placed such that it is located directly on the interface of the base of a light coupling optic or dispersion prism. Alternatively, a glass slide may be optically matched to the prism, a the sample located on the base of the slide. Total internal reflection then occurs at the base of the slide.

Typically, the objective lens and external light source are fixed in the lab frame and the sample on whic the light coupling optic or prism is fixed is scanned in a plane perpendicular to the objective lens axis. T prism therefore moves relative to the objective lens and the light source. Conventionally a45 or 60 dispersion prism is used, but to obtain light beams incident on the base of the prism at angles close to an greater than the critical angle, the light must usually be incident on the input face of the prism at off nor angles of incidence to achieve refraction of the beam at the air/glass interface. The deviation of the bean causes the reflection footprint at the base of the prism to walk db as the prism is translated dx towards o away from the light source. In a limiting case light propagating parallel to the prism base will be refract such that the footprint at the prism base moves equally and in the same direction as the prism(db/dx-0).

In this case the footprint moves dx in the lab frame and the illuminated area moves rapidly away from th imaging lens as the sample is scanned.

<Desc/Clms Page number 3>

It is an object of the present invention to obtain a footprint which is static in the laboratory frame of reference, defined by the objective lens and light source, such that the area illuminated at the glass/aque interface does not move away from the optical axis of the lens as the sample and prism are scanned.

According to the present invention, a scanning apparatus comprises:- a light source for generating a ligh beam; and an angular prism coupled to a sample at a base interface; characterised in that the base angle of the prism satisfies the equations:-(cos2#c - sin2#p) (tan#1 sin#p +cosOp) = 1 cos8 cos (#p +/- # and r sinAi =np sin(Op-Oc) whereinAc is the coupling angle required for light incident at the base interface o the prism, Ei is the incident angle of the light beam on the prism, ni is the refractive index of the mediur at the interface where the light beam enters the prism andnp is the refractive index of the prism.

According to the present invention, a scanning method comprises the steps of:- generating a light beam;

<Desc/Clms Page number 4>

providing an angular prism in the path of the light beam, the prism being coupled to a sample at a base interface; and moving the prism and sample relative to the light beam; characterised in that the base angleOp of the prism satisfies the equations:- (cos2#c - sin2#p) (tan#i sin#p + cos#p) = 1 cos#c cos(#p + #c) and nisine)=np sin(#p-#c) whereinSc is the coupling angle required for light incident at the interface the base interface of the prism, Si is the incident angle of the light beam on the prism, ni is the refractive index of the medium at the interface where the light beam enters the prism andnp is the refractive index the prism.

It has been found that, if the prism satisfies the above criteria, a solution exists where the footprint of th light beam on the base of the prism walks in an equal and opposite direction to the prism's translation in the lab frame i. e. db =-dx. Therefore, the point where the light incident to the prism intercepts the prism base is fixed in the lab frame. This results in scanning of the sample without movement of the footprint with respect to a detector fixed in the lab frame and hence no loss of image integrity.

A solution is found wherein6p=t andF > = 0.

<Desc/Clms Page number 5>

Preferably, the apparatus comprises a total internal reflection microscopy apparatus and includes means detecting interaction of the sample at the base of the prism or optically matched slide with an evanescen wave formed by total internal reflection of the light beam at the base of the prism or at the base of a slid which is optically matched to the prism. Preferably, the method. according to the present invention includes the step of detecting interaction of a sample at the base of the prism with an evanescent wave formed by total internal reflection of the light beam at the base of the prism.

For light entering the prism from air, ni and therefore sin Si =np sin (Ep-Sc)
For total internal reflection to occur at the base interface6, must be greater than or equal to the critical angle for the interface ie sin-1 (ns/np)
WhereinnS is the refractive index of the sample medium.

In total internal reflection microscopy, it is preferable that the coupling angle is greater than but close to the critical angle as this maximises the penetration of the evanescent wave into the sample medium.

It is generally preferable thatSc is slightly above the critical angle because, although penetration of the evanescent wave is at a maximum at the critical angle, there will be a spread of angles within the beam and, to ensure total internal reflection of the entire beam it is preferable to haveSc slightly above the critical angle.

<Desc/Clms Page number 6>

This also accommodates minor variations in the refractive indices of the interfacial media.

For a quartz/water interface at the base of the prism, wherenp=1. 46, the critical angle of the base interfa is66 . A preferred value for0e would be68 . A unique solution is found wherein ei = 0, andep =68 .

A preferred embodiment of the present invention will now be described with reference to the accompanying drawings; in which:-
Figure 1 shows an apparatus for Total Internal Reflection Fluorescence Microscopy;
Figure 2 is a schematic showing the total internal reflection footprint walking with the displacement of t prism; and,
Figure 3 is a graph of db/dx against prism angle for a quartz/water base interface and a coupling angle of68 .

Figure 1 illustrates an apparatus for Total Internal Reflection Fluorescence Microscopy having a light source 1 which generates a beam 2 which is incident on a prism 3 and is totally internally reflected at th base interface of the prism 3. A sample is positioned adjacent to a base interface of the prism 3 and the evanescent wave interacts with the sample, producing fluorescence. The fluorescence passes through an objective lens 4 and is directed towards a CCD camera 5 by a mirror6, passing througha filter 7.

As shown in Figure 2, the light beam 2 is incident on the prism 3 at6 > to the normal, is refracted as it enters the prism at6r to the normal and is incident on the base of the prism 3 at a coupling angleSc, forming a footprint at the base interface. For total internal reflection to

<Desc/Clms Page number 7>

occur,#c must be at least the critical angle for the base interface. As the prism moves dx in the lab frame position3', the footprint walks db in the prism frame.

The magnitude of the differentialdl/db may be derived as follows:- Sine rule 1 = r or rcos#c=lsin#p (1) s (90-e,)sinep Cosinerule r2 = b2 +12 - 2blcos#p (2) (1) in(2)12sin2#p=cos2#c(b2 + 12 - 2blcos#p) quadraticin 1 12(cos2#c-sin2#p)-2blcos#pcos2#c+b2cos2#c = 0 solution to which is
EMI7.1
expand contents of the square root
EMI7.2
take out4b2 cos2#c common in the square root
EMI7.3
factorizing gives
EMI7.4
differentiating w. r. t. b

EMI7.5
further factorization gives
EMI7.6

<Desc/Clms Page number 8>

recognisingcos2#p -1 = -sin2#p and factorising
EMI8.1
recognising1-cosse = sinue and rooting the square dl = cos#c(cos#pcos#c + sin#psin#c) db (cos2#c - sin2#p) using trigonometric identity the differential simplifies to dl = cos#cos (#p + #c) db (cos2#c - sin2#p)
The magnitude of thedifferential dl/dx may be derived as follows:- Sine ruledl = dx sin(90-#p+#i) sin(1
#p-(90-#p+#i) Simplifies to dl sin(90-#i) - dx sin(90-#p+#i)dlcors bai = dx cos(#p dl = cos#pcos#1+sin#psin#i dx Cos ei dl/dx =cos#p+tan#isin#p
Recognising that a translation of the prism dx in the positive x direction results in a displacement db of
footprint in the negative direction and by using the chain rule-db = dl x db dx dx2e so db = -(cos#p +tan#isin#p) x (cos2#c -sin2#p) dx cos#ccos(#p+#c)

<Desc/Clms Page number 9>

whereSi = sinl (npsin (Sp-Sc) fromFresnel's equation, for light entering the prism from air, (ni. = 1).

For the footprint to remain stationary in the lab frame, it must walk in an equal and opposite direction in the prism frame to the prism's translation in the lab frame, i. e.db/dx =-1.

In total internal reflection microscopy, it is preferred that the coupling angle is greater than but close to critical angle as this maximises the penetration of the evanescent wave into the sample medium, and it is generally preferable that9c is slightly above the critical angle because, although penetration of the evanescent wave is at a maximum at the critical angle, there will be a spread of angles within the beam and, to ensure total internal reflection of the entire beam, it is preferable to have 6 slightly above the critical angle.

For a quartz/water interface at the base of the prism, wherenp=1.46, the critical angle of the base interfa is66 . A preferred value forSc would be68 . Figure 3 shows the degree of walking of the footprint with prism displacement as a function of the internal angleep of the prism for#c =68 . The prism angle requir for db/dx =- 1 is 68 and the light will be incident normal to the input surface of the prism, i. e. Si =0.

Claims of **WO03062897**

CLAIMS1. A scanning apparatus comprising:- a light source for generating a light beam; and an angula prism coupled to a sample at a base. interface; characterised in that the base angleOp of the prism satisfi the equations:- (cos2#c - sin2#p) (tan#i sin#p + cos#p) = 1cos6 cos (ep+/-ex) and ni sinai =np sin(8p-6) wherein#c is the coupling angle required for light incident at base interface of the prism,01 is the incide angle of the light beam on the prism, ni is the refractive index of the medium at the interface where the light beam enters the prism and np is the refractive index of the prism.

2. A scanning apparatus according to claim 1, whereinSp=Sc and#i= 0.

3. A total internal reflection microscopy apparatus according to claim 1 or 2, including means for detect interactionof. the sample at the base of the prism with an evanescent wave formed by total internal reflection of the light beam at the base of the prism.

4. A total internal reflection microscopy apparatus according to claim 3, wherein

<Desc/Clms Page number 11>

Sc # sin-1 (ns/np) and whereinnS is the refractive index of the sample medium.

5. A scanning method comprising the steps of:- generating a light beam; providing an angular prism in t path of the light beam, the prism being coupled to a sample at a base interface; and moving the prism an sample relative to the light beam; characterised in that the base angle Bp of the prism satisfies the equations:-(cos2#c - sin2#p) (tan#i sin#p +cosse)cosse cos(@p +/-qu) and ni sin6i = np sin(0p-@C) wherein <RTI c is the coupling angle required for light incident at the interface at the base interface of the prism,Si is the incident angle of the light beam on the prism, ni is the refractive index of the medium at the interface where the light beam enters the prism and np is the refractive index of the prism.

6. A method of total internal reflection microscopy according to claim 5, further comprising the step of detecting interaction of a sample at the base of the prism with an evanescent wave formed by total intern reflection of the light beam at the base of the prism.
